nature portfolio

https://doi.org/10.1038/s41596-021-00544-y

Supplementary information

Comprehensive analysis of glycosphingolipid glycans by lectin microarrays and MALDI-TOF mass spectrometry

In the format provided by the authors and unedited

SUPPLEMENTARY INFORMATION

Comprehensive Analysis of Glycosphingolipid Glycans by Lectin Microarrays and MALDI-TOF Mass Spectrometry

Haoqi Du[†1], Hanjie Yu[†1], Fuquan Yang[2], Zheng Li[1*]

[1]Laboratory for Functional Glycomics, College of Life Sciences, Northwest University, Xi'an 710069, China

[2]Laboratory of Protein and Peptide Pharmaceuticals & Laboratory of Proteomics, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

†These authors contributed equally to this work.

***Corresponding Author**

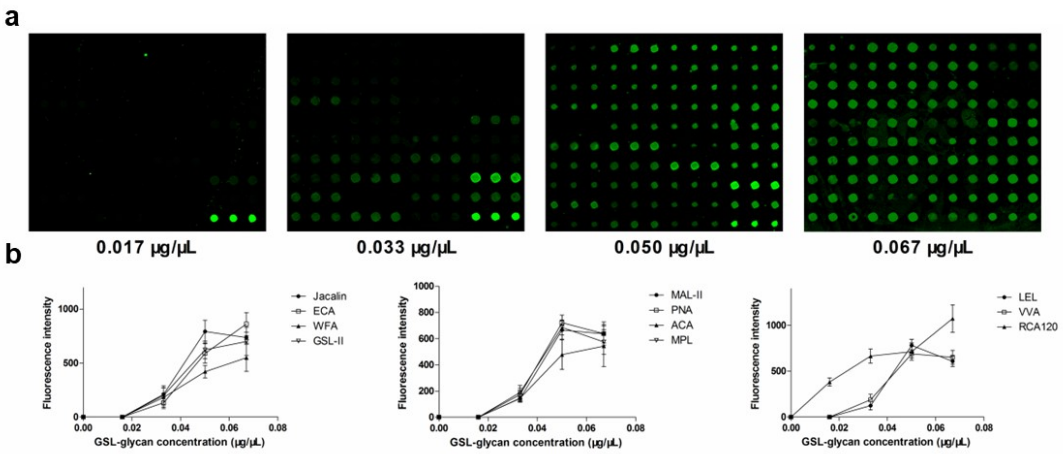
Prof. Dr. Zheng Li, Laboratory for Functional Glycomics, College of Life Sciences, Northwest University, No. 229 Taibai Beilu, Xi'an 710069, China.

Tel: +86-29-88304104; Fax: +86-29-88303572

E-mail: zhengli@nwu.edu.cn

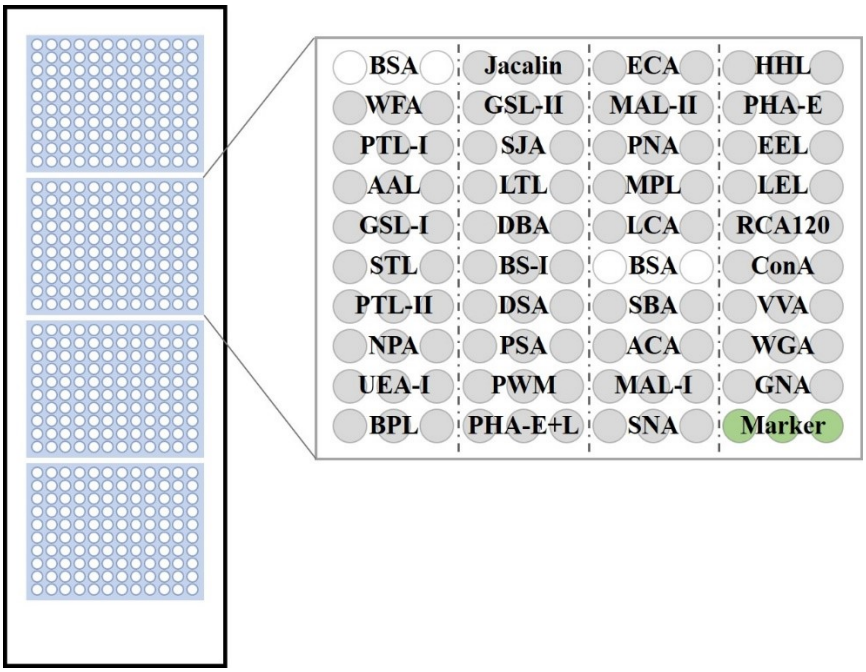
Table of Contents **Page number**

Supplementary Figures



Supplementary Figure 1: Different concentrations of GSL-glycans in the lectin microarray incubation system

a. Scan results of 0.017, 0.033, 0.050, 0.067 μg/μL of GSL-glycans added in the lectin microarray incubation system and **b.** fluorescent intensities of 11 lectins (e.g., Jacalin, MAL-II, LEL) with 0, 0.017, 0.033, 0.050, 0.067 μg/μL of GSL-glycans added in the lectin microarray incubation system. (This figure was previously published as Figure S4 of Du, H. et al. Analysis of glycosphingolipid glycans by lectin microarrays. Anal Chem 91, 10663-10671 (2019). This figure is reproduced with permission.)



Supplementary Figure 2: Layout of a lection microarray

Lectins were printed in triplicates in each of four matrixes on a slide. Detail of each lectin's information is described in Table 1.

Supplementary Tables

Supplementary Table 1

Glycopattern in HL-7702, MHCC97L, MHCC97H, and HCCLM3 cell lines by the lectin microarray analysis based on data of 37 lectins giving significant signal. (This table was previously published as Table S2 of Du, H. et al. Analysis of glycosphingolipid glycans by lectin microarrays. Anal Chem 91, 10663-10671 (2019). This table is reproduced with permission.)

Lectin	HL-7702	MHCC97L	MHCC97H	HCCLM3
Jacalin	0.060±0.009	0.041±0.003	0.043±0.002	0.048±0.003
ECA	0.044±0.001	0.036±0.011	0.026±0.001	0.056±0.011
HHL	0.019±0.007	0.037±0.005	0.041±0.002	0.004±0.002
WFA	0.033±0.001	0.021±0.001	0.027±0.001	0.051±0.017
GSL-II	0.055±0.005	0.048±0.005	0.055±0.008	0.083±0.012
MAL-II	0.008±0.002	0.032±0.004	0.025±0.008	0.011±0.001
PHA-E	0.011±0.011	0.021±0.002	0.012±0.003	0.030±0.006
PTL-I	0.006±0.003	0.016±0.001	0.020±0.002	0.004±0.001
SJA	0.004±0.004	0.008±0.001	0	0.038±0.005
PNA	0.006±0.004	0.017±0.003	0.011±0.002	0.023±0.002

EEL	0.025±0.011	0.037±0.007	0.049±0.011	0.005±0.002
AAL	0.141±0.045	0.076±0.025	0.062±0.013	0.035±0.002
LTL	0.006±0.001	0.015±0.002	0.019±0.003	0.021±0.002
MPL	0.014±0.006	0.028±0.002	0.031±0.006	0.011±0.001
LEL	0.031±0.001	0.029±0.007	0.030±0.004	0.034±0.004
GSL-I	0.011±0.003	0.028±0.001	0.012±0.002	0.035±0.001
DBA	0.015±0.007	0.024±0.009	0.023±0.006	0.040±0.002
LCA	0.062±0.021	0.033±0.016	0.034±0.010	0.046±0.007
RCA120	0.039±0.006	0.037±0.005	0.038±0.004	0.016±0.004
STL	0.037±0.005	0.031±0.004	0.034±0.003	0.042±0.007
BS-I	0	0	0	0.003±0.002
ConA	0.071±0.006	0.043±0.013	0.097±0.008	0.049±0.007
PTL-II	0.017±0.002	0.025±0.010	0.023±0.018	0.013±0.002
DSA	0.014±0.003	0.021±0.004	0.034±0.002	0.004±0.001
SBA	0.008±0.005	0.010±0.003	0	0.035±0.001
VVA	0.050±0.003	0.031±0.011	0.034±0.002	0.061±0.005
NPA	0.019±0.002	0.029±0.006	0.024±0.008	0.011±0.003

PSA	0.004±0.002	0.006±0.001	0.025±0.002	0.007±0.002
ACA	0.065±0.011	0.039±0.002	0	0.036±0.013
WGA	0.018±0.002	0.023±0.001	0.023±0.004	0.025±0.006
UEA-I	0	0	0	0.002±0.000
PWM	0.023±0.005	0.034±0.011	0.036±0.015	0.018±0.002
MAL-I	0.017±0.001	0.036±0.010	0.027±0.011	0.031±0.002
GNA	0.011±0.006	0.022±0.002	0.024±0.007	0.017±0.001
BPL	0.021±0.004	0.031±0.005	0.031±0.008	0.008±0.001
PHA-E+L	0.004±0.002	0.007±0.002	0	0
SNA	0.032±0.004	0.032±0.003	0.029±0.004	0.047±0.003

Supplementary Table 2

Fold changes of glycopatterns of GSLs from MHCC97L, MHCC97H, and HCCLM3 cell lines compared with HL-7702 cell line. (This table was previously published as Table S3 of Du, H. et al. Analysis of glycosphingolipid glycans by lectin microarrays. Anal Chem 91, 10663-10671 (2019). This table is reproduced with permission.)

Lectin	Specificity	HCC/Normal Ratio[a]		
		MHCC97L	MHCC97H	HCCLM3
Jacalin	Galβ1-3/1-4GalNAc, GlcNAcβ1-3GalNAc	-	-	-
ECA	Galβ1-4GlcNAc	-	0.596**	-
HHL	α1-3/1-6Man, Galα1-3Galβ1-4GlcNAc	1.975**	2.201**	0.193***
WFA	GalNAcα/β1-3/6Gal	0.632***	-	1.519**
GSL-II	αGalNAc, αGal	-	-	1.509***
MAL-II	Siaα2-3Gal	3.996***	3.118**	-
PHA-E	Galβ1-4GlcNAc, biantennary *N*-glycan	1.917***	-	2.742**
PTL-I	GalNAc, GalNAcα1-3Gal, GalNAcα1-3Galβ1-3/1-4Glc	2.639***	3.196**	-
SJA	Terminal GalNAc/Gal	2.052**	-	/***
PNA	Galβ1-3GalNAc, GlcNAcα1-3/1-4Gal, GalNAcβ1-4GlcNAc	2.740***	1.814***	3.693**
EEL	Galα1-3(Fucα1-2)Gal	-	2.007**	0.209***

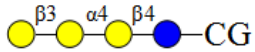
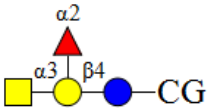
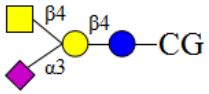
AAL	Fucα1-3Galβ1-4GlcNAc, Fucα1-6GlcNAc	0.536***	0.442**	0.246**
LTL	Fucα1-2Gal, Fucα1-3GlcNAc	2.414*	2.955*	3.384***
MPL	Galβ1-3GalNAc, Galβ1-3GlcNAc, GalNAc	2.017**	2.270*	-
LEL	(GlcNAc)n, poly-LacNAc	-	-	-
GSL-I	αGal, αGalNAc	2.496***	-	3.123***
DBA	GalNAc, GalNAcα1-3(Fucα1-2)Gal	1.620**	1.550*	2.732**
LCA	Fucα1-6GlcNAc	0.528***	0.553***	-
RCA120	Gal, Galβ1-4GlcNAc, Galβ1-3GlcNAc	-	-	0.419**
STL	Core GlcNAc, oligosaccharide containing GlcNAc	-	-	-
BS-I	Galα1-3Gal	-	-	-
ConA	Manα1-6(Manα1-3), terminal GlcNAc, terminal Gal	0.597***	-	-
PTL-II	Gal	-	-	-
DSA	β-GlcNAc, Galβ1-4GlcNAc	1.544**	2.505***	0.323***
SBA	α-/β- terminal GalNAc, (GalNAc)n	-	0***	4.398***
VVA	GalNAc, GalNAcβ1-3Gal, Galβ1-3/1-4GlcNAc	0.620***	-	-
NPA	High mannose	1.546***	-	0.604***
PSA	Fucα1-6GlcNAc, Fucα1-3GlcNAc	-	/**	-

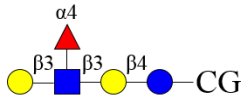
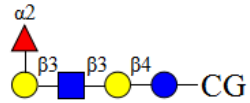
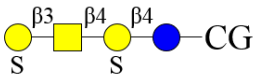
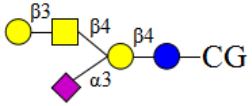
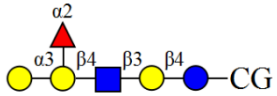
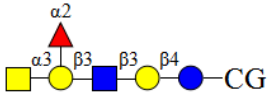
ACA	Galβ1-3GalNAc	0.603***	0***	0.561***
WGA	poly-Sia, Terminal GlcNAc	-	-	-
UEA-I	GAL	-	-	-
PWM	GlcNAc, Galβ1-4GlcNAc	-	1.554***	-
MAL-I	Sia2-3Gal, Galβ1-4GlcNAc	2.102***	1.602**	1.841**
GNA	α1-3/1-6Man Man, Galα1-3Galβ1-4GlcNAc	2.000**	2.174*	1.578**
BPL	Galβ1-3/1-4GalNAc	-	-	-
PHA-E+L	Tri- and tetra-antennary complex-type *N*-glycan	-	-	-
SNA	Siaα2-6Gal	-	-	-

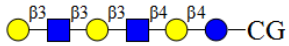
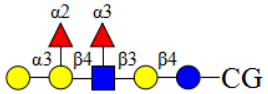
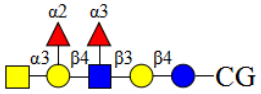
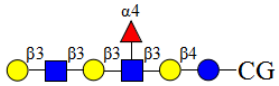
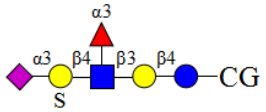
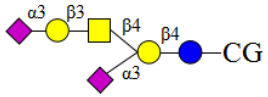
[a] The ratios of each lectin were calculated by comparing NFIs of different HCC cell lines with NFIs of HL-7702 cell line, respectively. -, no significant difference; /, the denominator of the fold-change was zero. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

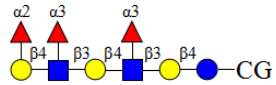
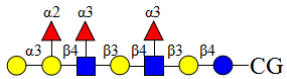
Supplementary Table 3

Proposed structures for GSL-glycans from HCC and HL-7702 cell lines based on MALDI-TOF/TOF-MS and lectin microarrays. (This table was previously published as Table S4 of Du, H. et al. Analysis of glycosphingolipid glycans by lectin microarrays. Anal Chem 91, 10663-10671 (2019). This table is reproduced with permission.)

No.	Calculated m/z	Predicted Components	Proposed Structures	Ion	Motif [a]	Relative Intensities of GSL-glycans			
						HL-7702	MHCC97L	MHCC97H	HCCLM3
1	706.229	Hex4-CG	Galβ1-3Galα1-4Galβ1-4Glcβ:CG	H^+		1.668%	4.570%	ND	ND
	728.211			Na^+		4.420%	3.765%	3.234%	4.016%
2	731.261	Hex2HexNAc1dHex1-CG	GalNAcα1-3(Fucα1-2)Galβ1-4Glcβ:CG	H^+		2.238%	2.262%	2.615%	ND
3	747.256	Hex3HexNAc1-CG	Galβ1-3GlcNAcβ1-3Galβ1-4Glc:CG	H^+		2.029%	1.039%	0.971%	1.435%
4	876.298	Hex2HexNAc1NeuAc1-CG	GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ:CG	H^+		4.184%	5.859%	8.119%	6.069 %

5	893.314	Hex3HexNAc1dHex1-CG	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+		2.570%	1.593%	1.676%	1.350%
			Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+					
6	929.151	Hex3HexNAc1S2-CG	SulfGalβ1-3GalNAcβ1-4SulfGalβ1-4Glcβ:CG	Na^+		1.946%	1.802%	1.605%	ND
7	980.309	Hex2NeuAc1NeuGc1-CG	NeuGcα2-8NeuAcα2-3Galβ1-4Glcβ:CG	H^+		ND	0.762%	ND	1.936%
8	1038.351	Hex3HexNAc1NeuAc1-CG	Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ:CG	H^+		3.310%	4.780%	10.943%	9.344%
9	1055.366	Hex4HexNAc1dHex1-CG	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+		2.468%	1.630%	1.696%	2.189%
10	1096.393	Hex3HexNAc2dHex1-CG	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+		ND	ND	ND	2.295%
11	1112.388			H^+		ND	ND	ND	2.765%

12	1134.370	Hex4HexNAc2-CG	Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ:CG	Na^+		ND	0.958%	ND	ND
13	1201.424	Hex4HexNAc1dHex2-CG	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+		ND	ND	ND	2.424%
14	1242.451	Hex3HexNAc2dHex2-CG	GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+		ND	ND	ND	2.075%
15	1280.428	Hex4HexNAc2dHex1-CG	Galβ1-3GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glcβ:CG	Na^+		0.682%	ND	ND	ND
16	1286.348	Hex3HexNAc1NeuAc1S1dHex1-CG	NeuAcα2-3SulfGalβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ:CG	Na^+		ND	ND	ND	1.604%
17	1329.447	Hex3HexNAc1NeuAc2-CG	NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ:CG	H^+		0.570%	ND	0.755%	1.105%

18	1550.562	Hex4HexNAc2d Hex3-CG	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1 -3Galβ1-4Glcβ:CG	H^+		ND	ND	0.707%	0.313%
19	1712.614	Hex5HexNAc2d Hex3-CG	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ:CG	H^+		ND	ND	1.288%	0.438%

[a] Monosaccharides are represented according to MS-tools from the GlycoWorkbench software (Glc, blue circle; GlcNAc, blue square; Gal, yellow circle; GalNAc, yellow square; Fuc, red triangle; NeuAc, purple diamond; NeuGc, white diamond). ND, not detected in the sample. CG, cyanomethyl glycosides residue.